Fine scale breeding structure in grey seal colonies is not based on kinship

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Abstract

Microsatellites were used to investigate fine-scale spatial and temporal genetic structure of a grey seal breeding colony, using samples collected throughout the colony (1997) and at a fine scale (2000-2002). Behaviour of breeding seals on North Rona, including philopatry and high breeding site fidelity, suggested female kin may cluster together. However, low F_{ST}-scores indicated no genetic differentiation between the major breeding aggregations, categorised as regions in the colony. Nevertheless, the pairwise relatedness of mothers within regions was significantly higher than the relatedness of mothers between regions. There was evidence of kin clustering within only one region in the colony. Therefore, sustained philopatry appears sufficient to produce differences in relatedness at a within-colony scale (> 500 m), but not at finer scales. However, within one region, mothers' pairwise relatedness decreased significantly with increasing distance between pupping sites. Pairwise relatedness of neighbouring females within this region was also higher than expected. Conversely, in the other regions mothers that were considered likely to have social interactions, based on their spatial and temporal proximity, were not significantly more related to each other than random. This suggests the social associations of mothers on North Rona detected previously are unlikely to be influenced by kin selection.

Key Words: genetic structure, microsatellites, grey seal, *Halichoerus grypus*, relatedness, kin clustering

Introduction

The spatial and temporal distribution of kin within a population can influence social behaviour, mating patterns and populations dynamics. A non-random distribution of kin may make interactions among close relatives common, allowing social behaviours that increase inclusive fitness to evolve through kin selection (reviewed in Hughes 1998; Ross 2001). Clustering of kin within a population can also increase the probability of inbreeding, and thus the possibility of inbreeding avoidance behaviour (Sugg *et al*. 1996). Therefore, knowledge of kin distributions within a population can help to understand and predict behavioural processes, and their consequences for individual fitness and population processes.

Natal dispersal is often sex biased, and in mammals males tend to be the dispersing sex (Greenwood 1980), with the result that many mammalian social groups are composed of female kin (e.g. Surridge *et al*. 1999; Kerth *et al*. 2000). While clustering of kin can occur passively, through high philopatry, it may also be an active process due to a preference for kin. Most detailed studies of kin associations have focussed on species that form cohesive social groups, but a few recent studies have examined the genetic structure of populations with more fluid social structures. For example, Coltman *et al*. (2003) found evidence of fine-scale genetic structuring in a population of wild Soay sheep *Ovis aries*, especially among females. Kin of some bird species nest close to each other within a colony as a result of extreme philopatry (e.g. great cormorants, *Phalacrocorax carbo sinensis*; Schjørring 2001) and a preference for familiar individuals (e.g. barnacle geese, *Branta leucopsis*; van der Jund *et al*. 2002).

Determination of the genetic relatedness of group members can help to assess whether kinship is likely to be influencing behaviour. For example, genetic analyses of cooperative breeding groups has often confirmed that they are composed of relatives which can, in some instances, explain helping behaviour (e.g. Russell & Hatchwell 2001). In species where kin hold neighbouring territories, kin-biased behaviours have been observed and reproductive successes of kin are greater

than the average (e.g. male red grouse, *Lagopus lagopus scoticus*, Piertney *et al*. 1999; female voles, *Microtus spp*, Lambin & Yoccoz 1998; Pusenius *et al*. 1998).

Phocids (true seals) often display high levels of philopatry and site fidelity to breeding colonies (Testa 1987; Baker *et al*. 1995; Pomeroy *et al*. 2000a; Härkönen & Harding 2001). However, previous studies have not found evidence for kin clustering within these temporary breeding assemblages (Perry *et al*. 1998; Schaeff *et al*. 1999; Pomeroy *et al*. 2001). Schaeff *et al*. (1999) found no correlation between the degree of relatedness of lactating female harbour seals *Phoca vitulina* and the time spent in the same group. Additionally, no genetic structure was apparent between female grey seals pupping on different beaches within island colonies (Perry *et al*. 1998). The latter studies used relatively small sample sizes and multilocus DNA fingerprinting, which is less useful than microsatellites for resolving relatedness (Bruford *et al*. 1992). However, a preliminary study of a larger grey seal breeding colony using microsatellites also found no evidence of fine-scale kin clustering in female seals, but rather found a gradation of relatedness such that individuals that were more highly related to the colony as a whole tended to occupy central positions within the colony (Pomeroy *et al*. 2001).

Nevertheless, the North Rona grey seal breeding colony has several characteristics that suggest that fine-scale genetic structuring based on kinship may exist. There is little evidence of effective dispersal in either sex, with pups born on North Rona returning there to breed as adults (Allen *et al*. 1995; Pomeroy *et al*. 2000a), a feature repeated at another breeding colony, the Isle of May on the east coast of Scotland (Pomeroy *et al* 2000a). There is also evidence that at least some females return to pup near the site within the colony where they were born (Pomeroy *et al*. 2000a). Females are also highly faithful to the site where they bred in the previous season (median distance moved between seasons = 39 m; Pomeroy *et al*. 2005). If philopatry occurred at the same scale, then associations of close kin could occur in the absence of significant immigration. Within this moderately polygynous mating system, males are also site faithful and long-lived (particularly

males that have greater reproductive success; Twiss *et al*. 1994), which could contribute to local similarity of genotypes. Individual females also generally return to breed within a few days of previous pupping dates (Pomeroy *et al*. 1999). Although the breeding season lasts for approximately ten weeks each year, individual females suckle their pups for an average of only 17 days before returning to sea (Pomeroy *et al*. 1999).

This high fidelity to both breeding site and time of breeding in different years can result in re-occurrence of inter-annual associations between grey seal mothers in the breeding colony (Pomeroy *et al*. 2005). This may be promoted on North Rona as mothers there usually remain close to their pups after birth and therefore to other neighbouring mothers as well, maintaining proximity. However some females also form active associations between years, implying a more complex social structure than had been previously recognised (Pomeroy *et al*. 2005). Detailed knowledge of kinship patterns within the colony is crucial in interpreting any observed social structure of grey seals on North Rona.

We investigated fine-scale genetic structuring and kin associations among female grey seals breeding in the North Rona colony. First, we assessed the extent of genetic structuring between distinct breeding aggregations within the colony, which were categorised as regions separated by c.800m. Since breeding females are usually faithful to these areas within the colony (Pomeroy *et al*. 1994), we predicted that there would be evidence for genetic differentiation between regions. Second, we examined patterns of relatedness between regions and within regions. Third, we tested the prediction that females that were likely to have social interactions, due to their spatial proximity and similar time of pupping, were more related to each other than to randomly selected mothers in each region.

Materials and Methods

Sampling

This study was conducted on North Rona (59°06´N, 05° 50´W), an uninhabited island lying about 75 km N.N.W. of Cape Wrath, Scotland. The breeding season on North Rona lasts from late September to late November with pup production reaching a peak in early to mid October. The majority of seals breed on the low-lying Fianuis peninsula (Figure 1). The peninsula is mostly cliff-bound and seals come ashore via access gullies that lead to the sea. The peninsula was divided into three breeding aggregations, or regions of roughly similar usable area, known as Fianuis North (FN) Fianuis South (FS) and Study Area (SA). These regions have been identified previously based on the location of access gullies to the sea and other topographical features that, in the main, effectively influence the distribution of pupping females (Figure 1; Pomeroy *et al*. 1994).

INSERT FIGURE 1 ABOUT HERE

Tissue samples were collected from grey seal mothers throughout the 1997, 2000, 2001 and 2002 breeding seasons. Females were recognised between breeding seasons by brands, tags, or pelage markings (Redman 2002). Thus a female was only skin sampled once but was included in analyses in all years she was observed in the colony with a pup. Samples taken in 1997 were collected from mothers throughout the breeding colony to assess colony-scale grouping. A total of 94 mothers were sampled from the FN region and 151 from the FS region (Figure 1). In all other years, mothers were sampled only from within the SA region to assess fine-scale grouping within areas monitored closely by observers on a daily basis. Samples obtained from 2000 on were taken opportunistically to identify as many occupants of these areas as possible without disturbing the structure of the breeding groups (2000 n = 73, 2001 n = 136, 2002 n = 121). Since approximately 1100 pups are born annually on North Rona, with approximately 450 born in SA (C. Duck pers. comm.), the sample we took only represents a small proportion of the mothers. Pups of an

additional 163 mothers sampled for a separate study were used here to check the accuracy of relatedness estimates.

Each sampled mother's location (to within a 10m x10m grid cell) was recorded in the field on a detailed geo-rectified map overlaid with a 10 m interval Ordinance Survey grid (Twiss *et al*. 2001). Either the observed pupping location of the mother or her location when first seen with a pup was recorded, as observed from hides overlooking the colony. In the few cases (3) where the pup was older than 10 days when the mother was first identified, sampling location was used.

Pupping date could be recorded directly if mothers were seen giving birth or had been observed the previous day without a pup. Otherwise, pupping date was estimated from the sampling date by subtracting the estimated day age of the pup. Pup ages were divided into five stages using a standard classification system based on size and developmental characteristics (after Kovacs & Lavigne 1986). If females were observed over longer periods, pup age could be estimated more accurately by including information about the length of time a pup remained at each stage.

The distribution of sampled mothers differed spatially and temporally between years in SA. This was due to both the actual distribution of mothers differing between years and differences in sampling, since the dataset represented only a subset of females present in the colony. To investigate the distribution of mothers, locations of all females in SA were recorded each day on detailed geo-rectified maps overlaid with a 10 m interval Ordinance Survey grid. These were entered into an ARC-INFO GIS of the North Rona colony (Twiss *et al* 2000, 2001, 2006). The number of females using each cell was estimated by taking the cumulative number of females present (measured daily) and dividing by 18, the average length of stay of mothers, in days. For each year, the season was defined as the first day data were obtained to the last day a pupping date was recorded. The proportion of mothers that was genotyped within each 10m x10 m grid cell was then plotted to represent an index of sampling intensity. Note that pupping dates of genotyped

mothers in SA were also compared between years using Kruskal-Wallis tests, as the data were not normally distributed.

Microsatellite genotyping

Skin samples were taken as tissue biopsies under Home Office licence (as described in Allen *et al*. 1995; Worthington Wilmer *et al*. 1999). Samples were stored in 20% DMSO or 96% ethanol and frozen at –20 °C until proteinase digestion and DNA extraction following standard phenol/chloroform protocols (Sambrook *et al*. 1989). Individuals were typed at up to 11 microsatellite loci (Table 1). These included nine that had previously been used with grey seals (prefixes Hg and Pv; Allen *et al*. 1995; Worthington Wilmer *et al*. 1999) and two not previously used (Hl15 and Lc6, Davis *et al* 2002). Primer sequences were taken from the references listed in Table 1 except for Hgdii, where a new reverse primer (AGG ACT CCT GCC ACT GAG AA) gave improved results. Each reaction contained 1 µl of DNA (~ 40 ng), 1 µl Promega 10x buffer, 1.25 - 1.75 mM of $MgCl_2$ (depending on the primer pair), 2 pmol of forward and reverse primers, 0.05 mM of each dNTP and 0.375 units of Taq polymerase (Promega), made up to 10 µl with sterile distilled water. Amplifications were performed in a PTC-100 Programmable Thermal Cycler, MJ Research, Inc. A 2 min denaturation step at 94 ºC was followed by 30 cycles of 10 seconds of denaturation at 94 ºC, 30 seconds of annealing at 52 – 60 ºC (depending on the primer pair Table 1) and 30 seconds of extension at 72 ºC and ending with 5 minutes at 72 ºC. Five additional cycles were added to the programme for Hl15 and Lc6 to increase yield. One primer from each pair was fluorescently labelled (Invitrogen) and PCR products run on a Beckman Coulter CEQ 8000XL system using the Fragment 3 programme.

INSERT TABLE 1 ABOUT HERE

For 96 samples taken in 1997, DNA was amplified and run on polyacrylamide gels following protocols outlined in Allen *et al*. (1995). Comparisons of 50 samples showed that the genotypes acquired using these different methods were directly comparable.

Since mothers could have been sampled more than once within or between seasons, all mothers genotyped for at least seven of the same loci were compared using the Identity function in CERVUS 2.0 (Marshall *et al*. 1998). The probability of an identical genotype occurring by chance in two different individuals at the seven least polymorphic loci was calculated following Paetkau & Stroebeck (1994). Duplicate samples were removed prior to any analyses. Tests for significant deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were implemented in GENEPOP 3.3 (Raymond & Rousset 1995). Null allele frequencies were estimated on CERVUS 2.0 (Marshall *et al*. 1998).

Estimating pairwise relatedness

An estimator for pairwise relatedness was chosen by assessing the means and standard errors of three commonly used estimators, (Queller and Goodnight 1989, Lynch and Ritland 1999 and Way 2002) against three sets of samples: 1) 125 mother-pup pairs, 2) 38 maternal half sibs which were checked using CERVUS that they did not have the same father and 3) 125 randomly selected female-female pairs from the set of all genotyped adult females. For all estimates of relatedness we used background population allele frequencies from 499 adult males and females sampled in the North Rona colony between 1997 and 2002. To achieve unbiased relatedness estimates, related individuals need to be excluded from the estimation of population allele frequencies. While this dataset contains an unknown number of related individuals, the large number of individual adults included in the sample should ensure a reasonable approximation of the population allele frequencies on North Rona. RELATEDNESS (Queller & Goodnight 1989) was chosen since it had the lowest variances for the three data sets (Van de Castelle *et al* 2001). We checked that the

estimates of the relatedness coefficient (R), with our microsatellite loci, gave scores similar to those expected; for mother pup pairs (expected R=0.5), half siblings (R= 0.25) and for random pairs of females (R = 0).

Spatial and temporal genetic analyses

Data from 1997 only were available to investigate the genetic structure of the whole colony, since only SA was sampled in 2000, 2001 and 2002. For the 1997 data analyses, the colony was first divided into the three regions (FN, FS and SA) and then FS-SA treated as one (Figure 1). The degree of genetic differentiation of the regions was estimated using F_{ST} and tested for significance using 10 000 permutations of individuals among regions. Allele frequencies were compared between regions in GENEPOP 3.3 (Raymond & Rousset 1995) and a Fisher exact test used to determine statistical significance. Average pairwise relatedness of females within regions was compared to those between regions using Mann-Whitney U tests. Since relatedness values were pairwise, significance was tested using 10 000 Monte Carlo randomisations in SPSS 11.0.

Population structure was also investigated using STRUCTURE Version 2 (Pritchard *et al*. 2000), which, estimates the most appropriate number of populations (K) required for interpreting the observed genotypes. Since the population on North Rona is recent and some geneflow is expected, we used no predefined population or locations of individuals. We assessed $P(K \mid X)$ both for the three sampling sites and for FS and SA combined and ran the programme three times at each setting to ensure consistency of results.. The programme assigns individuals to groups on the basis of their genotypes and provides the proportion of each seal's genome that originated in a particular sampling site. The model with the number of populations with the highest posterior probability and the proportional membership of the genome for each seal attributable to a sampling site is used to determine similarity among sampling sites. We set the burn in period to 50,000 iterations following convergence of log probability value and mean value of log likelihood .The probability estimates

were determined using 500 000 Markov Chain Monte Carlo iterations following the recommendation of Pritchard *et al* (2000).

Data from 1997, 2000, 2001 and 2002 were used to examine fine-scale spatial and temporal patterns of relatedness. The 1997 data were analysed separately for the FN, FS and SA regions Figure 1) in order to allow comparisons with other years. Distances between individuals were calculated as Euclidean distances. Regressions of matrices of pairwise relatedness and distance apart (in meters) were performed to test for spatial structuring. Following our definition, all mothers inside the same 10m x10 m grid cell and its eight immediately adjacent grid cells were chosen to represent neighbours. As mothers typically remain within 10 m of their pupping site (Pomeroy *et al*. 2005), mothers in more distant grid cells are unlikely to interact during the season. We compared the average pairwise relatedness of neighbours to the average relatedness of all other pairs of mothers within the specified region. For the above tests, statistical significance was tested using a procedure similar to a Mantel test with 10 000 permutations of locations among individuals. This maintained the observed spatial distribution information component of the data rather than redistributing animals in what may have been unrealistic locations.

Regressions of pairwise relatedness and difference in pupping date (in days) were performed to test for temporal aggregations of kin. Then, average pairwise relatedness of mothers pupping within nine days of each other was compared to those pupping more than nine days apart. As mothers suckle their pups for an average of 18 days, the nine day interval corresponds to pairs overlapping for half their breeding time. For temporal analyses, statistical significance was tested using 10 000 permutations of rows and columns of the date matrix, as in a Mantel test.

Standard errors for mean pairwise relatedness estimates were estimated by jackknifing over loci. All analyses described above were performed using SPAGeDi 1.1 (Hardy & Vekemans 2002) unless otherwise stated.

Results

Characterisation of microsatellite loci

Over the four years, 405 different mothers were genotyped. The 160 females sampled in 1997 were genotyped at 9 loci with 90% genotyped at all 9 loci. Of the 245 different mothers from 2000 to 2002 93% were genotyped for all 11 loci and 98.2% for at least 10 loci. An additional 66 female samples were genotyped but were found to be duplicates and were removed from the dataset. The error rate per locus was estimated from these re-sampled animals at 1% overall (ranging from 0 to 31%). Two loci (Pv9 and H115) had error rates of over 2%. Allelic dropout accounted for 14% of the errors. The likelihood of two individuals having identical genotypes for the seven least polymorphic loci was 2.0×10^{-7}, suggesting that identical genotypes were the result of re-sampling. Only one locus (Hg6.3, $p = 0.048$) showed significant deviation from HWE, but this was not significant after Bonferroni corrections for multiple comparisons. Two pairs of loci were in linkage disequilibrium (Hg6.1 & Hg8.9 and Hg8.10 & Lc6) but were not significant after Bonferroni corrections for multiple comparisons. The estimated frequency of null alleles was less than 0.025 for all loci.

Relatedness from known relationships

The mean (± SD) R-value of mother-pup pairs (expected value 0.50) was 0.49 ± 0.10 (n = 125) whereas the mean R-value of half-sibling pairs (expected value 0.25) was 0.27 ± 0.22 (n = 38). For randomly chosen female pairs (expected value 0.00) the mean R-value was –0.02 ± 0.16 (n = 125). Thus, relatedness estimates from microsatellite data reflected known genealogical relationships.

Representativeness and distribution of sampled females

There was some variation in the locations that seals used each year in SA. Mothers occupied the least number of grid cells in 2000 (101), the most in 2001 (187) (Figure 2), and 135 in 1997 and 133

in 2002. The number of densely occupied 10 m^2 grid cells (> 3 females) was greater in 2001 (n = 16) and 2002 (n = 14) than in 1997 (n = 7) and 2000 (n = 6). This suggests that the degree of clumping of females in the colony was not constant, although this is complicated by the fact that occupied cells represent the whole season. Sampling intensity was greatest in the southern part of SA in all years (Figure 2). In SA in every year at least one genotyped female was sampled in over half of the grid cells that were used in every year, and 66% of the occupied grid cells sampled in 2000 and 65% in 2001. As a result, most of the mothers present in areas of particular interest were sampled. Females in the crowded access gullies in the northeast SA were largely unsampled because of the high levels of disturbance this would have caused.

INSERT FIGURE 2 ABOUT HERE

The median pupping date of sampled mothers in SA was October 13th in all years except in 2000 when it was October 10th. There were significant differences between pupping dates of sampled mothers in SA between years (Kruskal-Wallis $\chi^2 = 9.94$, d.f. = 3, p = 0.009). Non-parametric post hoc tests (following Siegel & Castellan 1998) indicated the significant differences were between 2000 – 2001 and 2000 – 2002. This was probably due to earlier ending of sampling in 2000 (October 24th) than in 2001 and 2002 (November 6th and November 5th respectively).

Spatial and temporal genetic structure

There was no evidence of genetic differentiation within the North Rona colony when it was divided into two, FN and FS/SA ($F_{ST} = 0.001$, p = 0.230), or into three, FN, FS and SA ($F_{ST} = 0.002$, p = 0.160). Allele frequencies between regions were also not significantly different (FN and FS/SA, $\chi^2 = 25.05$, d.f. = 22, p = 0.295; FN, FS and SA, $\chi^2 = 30.61$, d.f. = 22, p = 0.104). The analysis of population structure run on STRUCTURE also identified K=1 as the most probable number of populations with the highest posterior probability on each run ($(P (K \mid X) > 0.999)$. Within the North Rona sampling sites when 3 sampling sites were specified one third (0.337-0.332) of the genotypes

from each sampling site was assigned to each of the three clusters indicating an absence of population structure between the sampling sites.. Similarly, when FN and FS/SA were specified as two sites one half of the genotypes from each sampling sites was assigned to the other one (0.52-0.48).

Next, kin clustering within the colony was examined. When the colony was divided into two regions (FN and FS+SA), mothers within regions were significantly more related to each other than mothers between regions (Figure 3; $Z = 2.80$, $N_1 = 15696$, $N_2 = 14194$, $p = 0.005$). However, when the colony was divided into three (FN, FS and SA) this relationship disappeared (Figure 3; $Z = -.350$, $N_1 = 10926$, $N_2 = 18964$, $p = 0.726$).

INSERT FIGURE 3 ABOUT HERE

INSERT TABLE 2 ABOUT HERE

Pairwise relatedness of mothers decreased significantly with increasing distance between pupping sites in FS in 1997 (Table 2). This was the only year or sub-region where a significant relationship between spatial distance and pairwise relatedness was detected. In 1997, average pairwise relatedness of neighbours was significantly higher than expected if mothers were randomly distributed in SA ($p = 0.031$) and FS ($p = 0.007$) only (Figure 4). However, only the FS result remained significant after Bonferroni corrections for multiple comparisons (i.e. at $p < 0.008$). Mean relatedness of neighbours within FS (0.09) approached that of first cousins (i.e. 0.125), and 22% (24/108) of neighbours in FS had relatedness equal to or greater than that of half-siblings (0.25). In all other years average pairwise relatedness of neighbours in SA was close to zero and was not significantly different from values expected for pairs selected randomly from the region (Figure 4).

INSERT FIGURE 4 ABOUT HERE

There was no significant relationship between pairwise relatedness of mothers and the time between their pupping dates for any region or year (Table 2). Average pairwise relatedness of mothers

pupping both within nine days of each other and more than nine days apart were not significantly different for any sub-region or year (Figure 5).

INSERT FIGURE 5 ABOUT HERE

Discussion

Genetic differentiation of regions

Since female grey seals in the UK are philopatric (Allen *et al*. 1995; Pomeroy *et al*. 2000a) and highly faithful to previous pupping sites (Pomeroy *et al*. 1994; 2005), we predicted that we would find evidence of fine scale genetic structuring of females within the North Rona breeding colony. However, no significant genetic differentiation was found between the major breeding regions, suggesting that some combination of gene flow within the colony or dilution of close kin relationships by incomers is sufficient to mask such a pattern. Gene flow may be caused by adult migrants moving between regions on a colony and between breeding colonies; both of these behaviours have been observed in UK grey seals (Pomeroy *et al*. 2000a; Redman 2002). Furthermore, females do not always give birth every year (Pomeroy *et al*. 1999) and in years when no pup is raised, may mate with males not sampled at or away from their usual breeding colony (Worthington Wilmer *et al*. 1999). Even in the absence of gene flow, the colony may appear panmictic because there has not been enough time for genetic differences to accumulate. The current North Rona breeding colony expanded after people ceased to inhabit the island, probably between 1844 and 1880 (Boyd *et al*. 1962). With a generation time of 16 years for grey seals (J. Harwood pers. arbo.), there has been very little time for any genetic structuring to appear. Therefore, it seems likely that both gene flow and the brief period of time since the establishment of separate breeding regions on the island may account for the lack of genetic structuring.

Our findings tend to mirror the lack of genetic differentiation found within grey and harbour seal colonies by other studies (Perry *et al*. 1998; Schaeff *et al*. 1999; Pomeroy *et al*. 2001). Although genetic differentiation was reported between the North Rona and Isle of May colonies

(Allen *et al*. 1995), which are separated by ~500 km, studies of grey seal colonies that are in closer proximity to each other have reported an absence of genetic divergence (e.g. east coast of Canada, Boskovic *et al*. 1996; Orkney Islands, Gaggiotti *et al*. 2002). The lack of genetic structuring between these colonies, as at North Rona, is probably due to both current and historic gene flow, as many of the populations have fluctuated in size or been recently founded. It would appear that other pinniped species display genetic differentiation between colonies separated at a similar scale (~ 500 km) (Goodman 1998; Palo *et al*. 2001).

Philopatry

The relatedness of mothers within the two main pupping regions of the colony (FN and FS/SA) was higher than the relatedness between them (Figure 3), suggesting that females tend to be natally site faithful within these two regions of the colony. No trends were observed when the colony was divided into three indicating that the relatedness of mothers between FS and the areas to either side was comparatively high. Additionally, within the SA and FN no significant kin clustering was detected in any year (Table 2). Thus the precision with which females return to their natal site does not generally appear high enough to create patterns of kin clustering within regions but was sufficient to produce a difference in relatedness between the north and south of the colony.

Philopatry has previously been estimated to be within 100 m from the few available marked females on North Rona (Pomeroy *et al*. 2000a). This level of philopatry would be unlikely to produce measurable kin clustering within regions, since they are each only a few hundred meters across, but could produce differences in relatedness between regions (Figure 1). Therefore, our results generally agree with those from the earlier tagging study. However, a larger mark-recapture study would help to determine the scale of philopatry more precisely. Further studies should also include information on age and sex, which have been shown to be important in assessing philopatry in other pinniped species (Baker 1995; Härkönen & Harding 2001). For example, using mark-

recapture techniques, northern fur seals (*Callorhinus ursinus*) were found to display a significant tendency to breed in their natal area within a colony, and this philopatry increased with age and was greater among females (Baker *et al*. 1995).

A high degree of philopatry is beneficial in many species because, in an unchanging habitat, individuals may get access to higher quality resources at their natal site (Schjørring 2001; Pärt 1991). Habitat quality varies in grey seal colonies, with preferred pupping sites being close to access to water with low levels of disruption by conspecifics (Pomeroy *et al*. 2000b; Twiss *et al*. 2000; 2001). However, while some topographic characteristics are constant, locations and sizes of pools of water and the distribution of females, and therefore also males, can change between years, altering the quality of sites. Females come ashore prior to pupping for an average of two days and may use this time to evaluate pupping sites (Pomeroy *et al*. 1994). However, females may also be unable to pup in their natal sites because of competition for high quality habitat. The most preferred sites in the colony are occupied first (Pomeroy *et al*. 2000b; Twiss *et al*. 2001) by mothers that may be older and of higher quality (Pomeroy *et al*. 1999; 2001; Twiss *et al*. 2000). Therefore, changing habitat quality and competitive ability may influence local site selection in the colony, decreasing philopatry and resulting in an absence of kin clustering.

While genetic structuring appeared absent in most of the colony there was evidence of kin clustering in FS (Table 2). This is the first time to our knowledge that kin clustering has been identified in a phocid colony. In this case, many neighbouring mothers had high relatedness values (> 0.25) and thus may be considered close kin. It is possible that kin clusters were detected in this region because of habitat structure. Mothers in FS typically cluster around one main area that allows good access to water. Having higher philopatry in this region may be more beneficial than in FN and the SA where habitat quality is less diverse. The number of seals occupying the area is also generally lower in FS, which could lead to less competition in this region and allow females to pup near their natal site. Unfortunately, only one year of data from FS was available with a moderate

sample size (n = 45 mothers). Data from additional years are needed to confirm that kin clustering regularly occurs in this sub-region and to investigate whether kin form long-term associations.

Temporal patterns of relatedness

There was no evidence that mothers gave birth at similar times to their relatives in any region, or year (Table 2; Figure 5). If mothers gained substantial benefits from interacting with relatives, then there would be some evolutionary pressure to pup at the same time during the breeding season. However, physiological differences in females may be a more important proximate factor in determining the date at which individuals give birth. In the UK, females are mated on the breeding colony in the autumn but implantation of the embryo is delayed until spring (Boyd 1984). It is thought that body composition may affect the individual timing of implantation (Boyd 1984). Furthermore, older, larger and higher quality females tend to pup earlier, suggesting pupping date, on N Rona at least, is determined by individual quality (Anderson & Fedak 1987; Pomeroy *et al*. 1999). Pupping date may also depend on a female's history, since those that do not breed in one year return to pup earlier in the subsequent year (Pomeroy *et al*. 1999). Hence, if pupping date is determined mainly by individual quality and previous reproductive history, pupping time for close relatives is less likely to be synchronous.

Effect of sampling bias

Overall, samples were representative of mothers pupping within the colony with a few exceptions. Specifically, certain locations within the SA were rarely sampled due the high level of disturbance this would have caused (Figure 2). Therefore, mothers in these locations could not be taken into account. Although sampling tended to be consistent, samples in certain years were sometimes biased towards specific times or places (e.g. few late breeding mothers were sampled in 2000).

There is a possibility that this may obscure patterns of genetic structure or produce spurious results. However, this is unlikely for the SA.

Role of relatedness in sociality

With the exception of those in FS, neighbouring mothers that were considered likely to have social interactions (on a spatial and temporal basis) were no more related to each other than expected (Figure 4 & 5). The general lack of fine-scale kin clustering found here suggests that pupping near kin may either not be feasible , due to competition with conspecifics, or may not be sufficiently advantageous for the females to seek them out. Consequently, the social structure of mothers on North Rona is unlikely to be influenced by kin selection. Instead, mothers may form associations based on other cues, such as cohort membership (Pomeroy *et al*. 2005). Although these relationships are unlikely to provide indirect fitness benefits, they may increase direct fitness or be maintained through reciprocity. Both of these factors are important in the maintenance of social behaviours in other species (e.g. Grinnell *et al*. 1995; Olendorf *et al*. 2004; Van horn *et al*. 2004).

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Table 1: Genotype data for each locus, including observed heterozygosity and the estimated frequency of null alleles.

Locus	Reference	Number of Alleles	Observed Heterozygosity	Annealing temp. (^{o}C)	Number of seals geotyped/ 405	Estimated frequency of null alleles
Hg3.6	Allen *et al*. 1995	8	0.791	58	402	-0.006
Hg4.2	Allen *et al*. 1995	10	0.672	58	393	-0.040
Hg6.1	Allen *et al*. 1995	6	0.600	62	399	0.014
Hg6.3	Allen *et al*. 1995	6	0.780	60	383	0.001
Hg8.9	Allen *et al*. 1995	11	0.864	58	401	-0.018
Hg8.10	Allen *et al*. 1995	10	0.778	60	393	0.009
Hgdii	Allen *et al*. 1995	8	0.687	56	399	-0.005
Pv9	Goodman 1997	7	0.777	52	396	0.019
Pv11	Goodman 1997	8	0.677	60	404	0.023
Hl15	Davis *et al.* 2002	17	0.871	56	309*	-0.004
Lc6	Davis *et al.* 2002	11	0.774	55	309*	0.022
Mean		9.3	0.755			

* out of 309 seals

Table 2: Regressions between the relatedness of pairs and both their distance apart and the time between pupping dates. Significant values are highlighted in bold. Sample sizes (n) are the number of individual mothers.

Year	**Sub-region**	**n**	**Euclidian distance (m) between pupping sites**		**Time (d) between pupping dates**	
			Slope	**p**	**Slope**	**p**
1997	SA	106	1.81×10^{-5}	0.553	1.73×10^{-4}	0.587
1997	FS	45	**-4.90×10^{-4}**	**0.006**	-1.99×10^{-3}	0.244
1997	FN	94	-5.99×10^{-5}	0.182	1.56×10^{-3}	0.952
2000	SA	73	-1.27×10^{-5}	0.899	1.90×10^{-3}	0.764
2001	SA	136	4.76×10^{-5}	0.607	9.34×10^{-5}	0.831
2002	SA	121	-1.07×10^{-5}	0.858	-6.12×10^{-4}	0.222

Figure Legends

Figure 1: The Fianuis peninsula, North Rona. The solid line indicates the division between the two main breeding regions (Fianuis North and Fianuis South/Study Area). Study Area is indicated by the dashed line. Stars indicate the locations of access gullies to the sea (modified from Pomeroy *et al*. 1994).

Figure 2: Sampling intensity and distribution of females pupping in each 10m x 10m grid cell in FS/SA. Only data from 2000 and 2001 are presented here, to provide an example of the differences between years. X and Y axes are OSGB eastings and northings respectively (181150-181530 (min-max easting) and 1032600-1032900 (min-max northing)). Blank cells represent sea; the remaining cells represent land with or without seals. Land containing no seals is set at a value of 0. Land containing seals where none were sampled has been given an arbitrary value of 10 to distinguish from the no seals. The remaining cells represent the actual sampling intensity.

Figure 3: Means ± SE of pairwise relatedness values of mothers within regions and sub-regions (white bars) and between regions and sub-regions (grey bars) when the colony was divided into two (FN and FS/SA) and three (FN, FS and SA).

Figure 4: Means ± SE of pairwise relatedness of neighbours (white bars) and all other pairs (grey bars).

Figure 5: Means ± SE of pairwise relatedness of mothers pupping within nine days of each other (white bars) and pairs pupping more than nine days apart (grey bars).

Figure 1

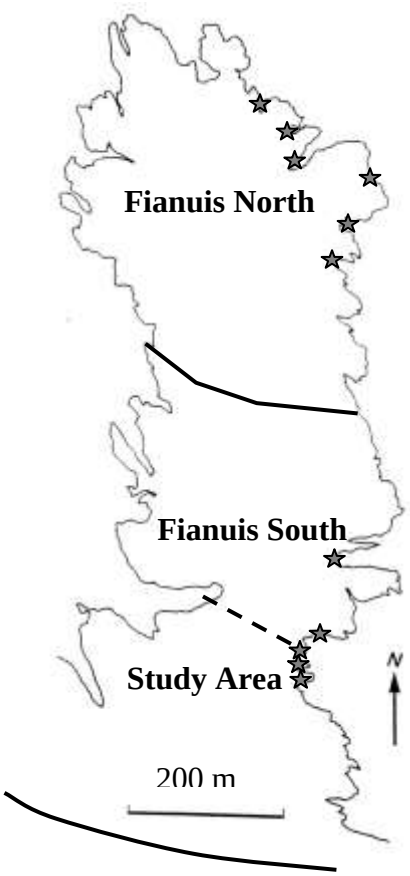


Fig 2

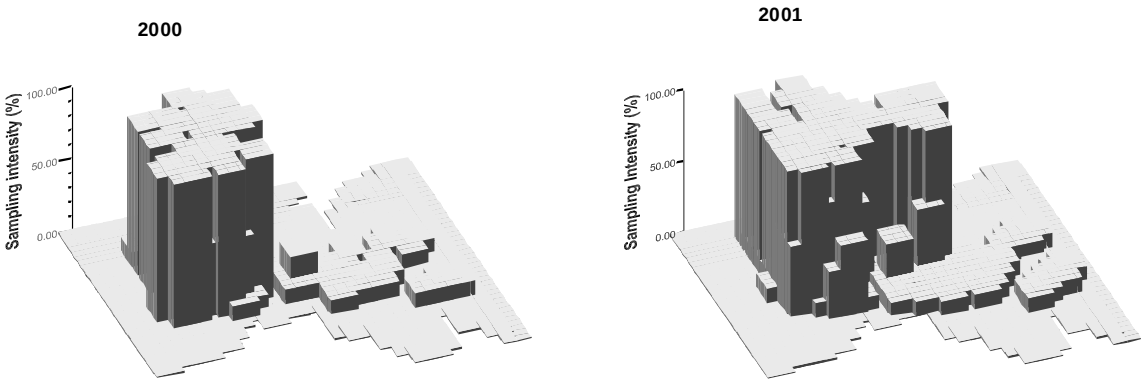

2000
Sampling intensity (%)
100.00
50.00
0.00
2001
Sampling Intensity (%)
100.00
50.00
0.00

Figure 3

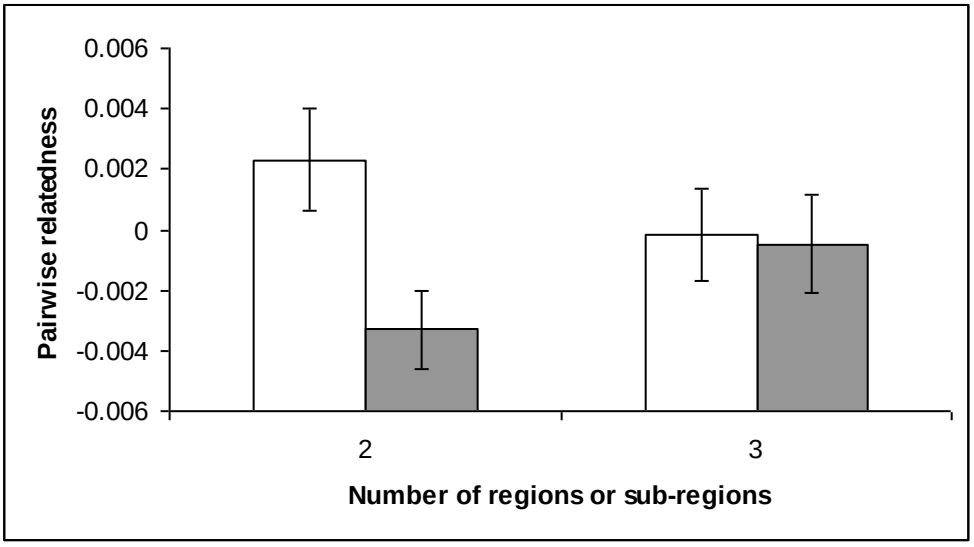

0.006
0.004
0.002
0
-0.002
-0.004
-0.006
Pairwise relatedness
2
3
Number of regions or sub-regions

Figure 4

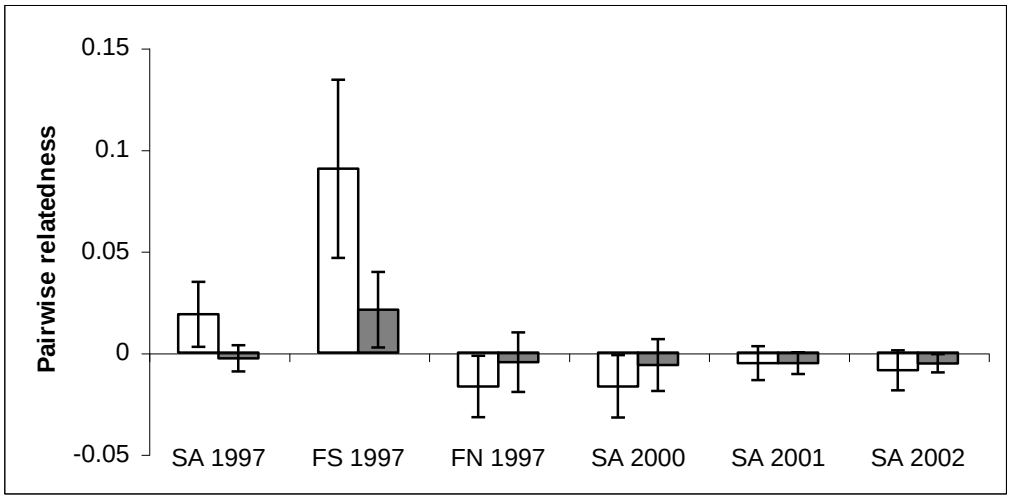
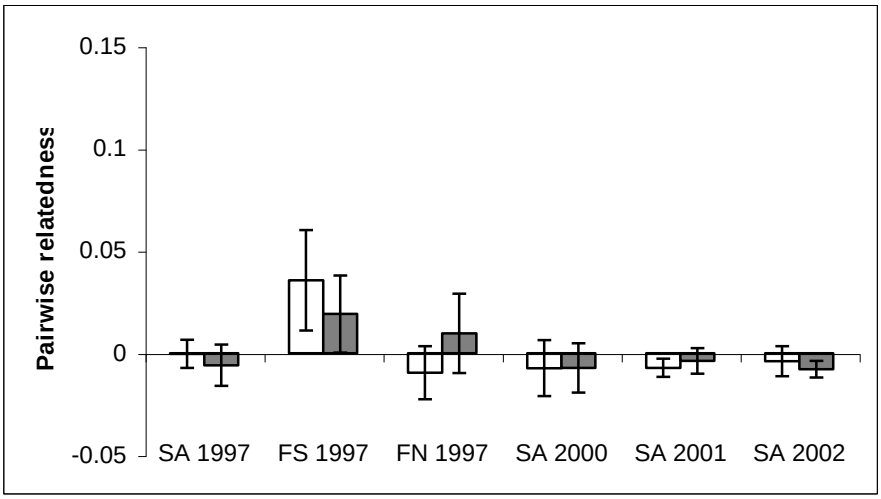

Pairwise relatedness
0.15
0.1
0.05
0
-0.05
SA 1997
FS 1997
FN 1997
SA 2000
SA 2001
SA 2002

Figure 5


0.15
0.1
0.05
0
-0.05
Pairwise relatedness
SA 1997
FS 1997
FN 1997
SA 2000
SA 2001
SA 2002